PATENT
Attorney Docket No.: A-54528-8/WHD

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Examiner: SMITH, L.
BERMAN, et al.	Group Art Unit: 1813
Serial No. 08/357,084	
Filed: 15 December 1994	
For: VACCINE BASED ON MEMBRANE BOUND PROTEINS AND PROCESS FOR MAKING THEM	

DECLARATION UNDER 37 CFR 1.131

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

We, Laurence A. Lasky and Phillip W. Berman declare as follows:

1. We are the applicants of the above-identified patent application. We are familiar with Claims 1, 2, 10, 13, 14, 17, and 20 through 23, which we have been informed are currently pending in the above application, and are the inventors variously of the subject matter they cover.

2. We are also familiar generally with the office action mailed on 18 April 1995 in this application wherein, inter alia, the claims stand rejected over certain publications.

EXHIBIT D

3. We further declare that, prior to at least July 1982, the invention as described and claimed in the present application was completed in this country as evidenced by the following:

4. Prior to July, 1982 the invention as described and claimed in the present application was conceived in this country. Prior to July 1982, said conception was coupled with due diligence to a subsequent reduction to practice in this country.

5. Appended DOCUMENT 1 includes pages 62-63, 79, 82 and 86 from notebook 668 of Ms. Sharon Apperson. These pages disclose that as part of Genentech's ongoing interferon project, Ms. Apperson maintained stocks of HSV-1 and HSV-2, routinely titering these viruses on various cell lines. Additionally, Ms. Apperson routinely performed plaque reduction assays with various interferons on cell lines infected with either HSV-1 or HSV-2. As disclosed at pages 62 and 86, the stock strain of HSV-1 maintained by Ms. Apperson was HZT and a mammalian cell line she routinely infected with this strain of HSV-1 was Hep 2. Dr. Lasky requested that Ms. Apperson provide him with Hep 2 cells infected with the HZT strain of HSV-1 as well as cells infected with HSV-2. As discussed below, Ms. Apperson provided Dr. Lasky with infected cells from which he and his technician, Mr. Donald Dowbenko, were able to isolate HSV-1 and HSV-2 DNA for their cloning studies. *See infra at 8, 9.*

6. Appended DOCUMENT 2 includes pages 52-53, 56, 60-63, 64-70, 75, 77, 81, 83, 85, 87, 90, 92 and pages 95-96 from notebook 1117 of Dr. Lasky. These pages disclose Dr. Lasky's attempts at isolating and cloning HSV-1 and HSV-2 DNA. Page 52 discloses Dr. Lasky's first attempt at isolating HSV-1 viral DNA from HSV-infected Hep-2 cells provided previously by Ms. Apperson. Additionally, experiments beginning at page 75 disclose the cloning of an HSV-1 gD encoding DNA fragment into an expression vector capable of transforming mammalian (COS) cells.

7. Appended DOCUMENT 3 includes pages 7, 9, 10-19, 23-23, 25-26, 29-33, 51, 54, 60, 61, 66, 68, 70, 71 and page 84 from notebook 1256 of Mr. Donald Dowbenko, a technician working under Dr. Lasky's direction and control. Experiments ending at page 31 disclose Mr. Dowbenko's successful isolation, cloning and sequencing of an HSV-1 gD encoding DNA fragment. Pages 9-10, and 16 disclose Mr. Dowbenko's successful cloning of a 6-7 kb Bam H1 fragment into Bam H1 digested pBR322 and subsequent subcloning of the correct gD encoding Sst-1 fragment into Sst-1 digested plasmid pFM3 yielding plasmid pJ 2.9. Mr. Dowbenko's experiments included isolating and cloning HSV-2 gD encoding DNA sequences. In addition, experiments disclosed on page 61 evidence work related to creating a mammalian expression vector capable, when introduced into CHO cells, of

producing a non membrane-bound (truncated) expression vector capable, when introduced into CHO cells, of producing a non membrane-bound (truncated) HSV-1 gD protein.

8. Appended DOCUMENT 4 contains pages 1, 3, 9, 14-17, 20-24, 26-27, 29-31, 33-34, 36, 38-45, 50, 56-57, 60, 65-67, 69-71, and 74 from notebook 1424 of Dr. Lasky. As disclosed on page 1, Dr. Lasky constructed a mammalian expression vector capable, when introduced into CHO cells, of producing a membrane bound HSV-1 gD protein. Furthermore, as disclosed on pages 14-17, Dr. Lasky constructed a mammalian expression vector capable, when introduced into and CHO cells, of producing a non membrane bound (truncated) gD protein. These plasmids were transferred to Dr. Christian Simonsen. Under Dr. Lasky and Dr. Berman's direction and control, Dr. Simonsen introduced these plasmids into Chinese hamster ovary (CHO) cells thereby creating mammalian cell lines producing HSV-1 gD protein. Pages 23-24, 26-27, 29-31, 33-34, 35, 38-45, 50, and 65 disclose Dr. Lasky's continued efforts at isolating and cloning HSV-2 gD DNA sequences.

9. Experiments beginning at page 56 of Lasky notebook 1424 include Dr. Lasky's investigation of the neutralizing effects of antibodies generated in rabbits immunized previously with CHO cells producing full-length membrane bound gD protein. The immunizing

CHO cell line, gD12, is discussed more fully below. These experiments indicate that as early as March, 1983, Drs. Lasky and Berman demonstrated successful in vitro neutralization using sera from gD12 immunized rabbits.

10. Appended DOCUMENT 5 includes pages 4, 9, 12, 21 and 24-28 from notebook 1915 of Dr. Lasky. These pages disclose Dr. Lasky's continued investigation into the neutralizing effects of antibodies generated against membrane bound gD expressing CHO cells (gD12). In addition, these pages show successful in vitro neutralization using sera derived from mice immunized previously with a truncated form of gD protein. The immunizing protein was obtained from a CHO cell line referred to as gD 10.2. This cell line will be discussed more fully below. *See infra at 13.*

11. As exemplified at page 4 dated June, 1983, Dr. Lasky performed a viral neutralization assay using sera obtained from 11 mice injected and boosted previously with the truncated (10.2) form of the gD protein. Dr. Lasky concluded that the gD 10.2 secreted product generates neutralizing antibodies to HSV-1. Serially diluted mouse sera were incubated with 40 plaque forming units (PFU) of HSV-1 for 1 hour at 37 C. Each dilution was applied to 40,000 Vero cells contained in each well of a 96 well microtiter plate. On page 21 this experiment was repeated in an in vitro experiment utilizing HSV-2 as the challenge

virus. The conclusion was the same, namely that antibodies to the truncated gD protect cells from a lethal HSV-2 challenge.

12. Page 9 discloses an experiment wherein Dr. Lasky measured the lethality of the herpes HSV-1 MacIntyre strain as well as the HSV-2 MS strain. In doing so, he was able to determine the proper dose for an in vivo challenge of mice immunized previously with either the membrane bound or truncated form of gD. At pages 24-28 Dr. Lasky performed in vivo neutralizing assays measuring protection against an HSV-1 infection in mice previously immunized according to animal protocol 527 discussed below. *See infra at 24, 26.* As the data indicates, mice previously immunized with cells producing membrane bound gD (gD12 cells) and mice immunized with the truncated gD protein (10.2) both elicited antibodies capable of neutralizing HSV-1 virus in vivo.

13. Appended DOCUMENT 6 includes pages 56, 59, 68, 72, 80, 84, 90-92 and 94 of notebook 915 of Dr. Christian Simonsen a scientist working under the direction and control of Drs. Lasky and Berman. As disclosed on page 59, Dr. Simonsen introduced the pgD DHFR plasmid previously constructed by Dr. Lasky into CHO cells deficient in the production of dhfr (dhfr$^-$). *See supra at* 8. Subsequently, at page 80, Dr. Simonsen chose two colonies producing membrane bound gD for subcloning. One of these colonies, gD12A

(gD12) was chosen for further study including amplification in the presence of methotrexate (MTX). Dr. Lasky's truncated gD construct (*See supra at* 8) was introduced into CHO dhfr- cells in January, 1983 as disclosed at page 91. Of the colonies arising from this transfection, one originally designated as 91.10.2 and later called simply 10.2 was chosen for further study.

14. Appended DOCUMENT 7 includes pages 1, 5, 8-9, 15 and 46 of notebook 1584 of Dr. Christian Simonsen. Dr. Simonsen's experiments include indirect immunofluorescent labeling of both gD12 and 10.2 CHO cells. These notebook pages further disclose metabolic labeling experiments using ^{35}S-methionine on both gD12 and 10.2 CHO cell lines producing gD protein. The labeled products were immunuprecipitated by Dr. Berman.

15. Appended DOCUMENT 8 includes pages 7-12, 21-22, 41-43, 47-57, 81, and 85-96 of notebook 1462 of Dr. Phillip Berman. These pages disclose a number of gD related experiments. Pages 41-43 and 48-51 disclose an example of Dr. Berman's successful immunoprecipitation of membrane bound gD produced by CHO cells transfected previously with Dr. Lasky's pgD DHFR construct. Based on these results, a particular membrane bound gD producing cell line was chosen (designated gD12). Furthermore, monoclonal antibody immunoprecipitation of membrane bound gD from gD12 CHO cells

allowed Dr. Berman to conclude that the protein possessed neutralizing determinants. See page 19 of Berman notebook 1585, discussed *infra at 17.* Moreover results presented on page 55 indicate that immunofluorescence experiments with CHO and gD12 showed fluorescent labeling of the membrane bound form of HSV-1 gD.

16. Experiments beginning on page 81 of this notebook disclose Dr. Berman's attempts at developing an enzyme- linked immunoadsorption assay (ELISA) based upon the binding of anti-HSV antibodies (from sera) to gD12 cells plated and chemically fixed to the wells of a 96 well microtiter tissue culture plate. Pages 91-96 disclose the usefulness of this assay in detecting antibody titers to gD from the sera of individuals known to posses anti-HSV-1 or anti-HSV-2 antibodies. Further, Berman concluded, at page 96, that "These results unambiguously demonstrate that mammalian cells (in this case CHO cells) transfected with DNA coding for a mammalian coat protein can be used as a diagnostic reagent to determine antibody titers to viruses".

17. Appended DOCUMENT 9 includes pages 1-19, 34-45, 48-53, 55-57, 60-61, 79-81, and 83-85 of notebook 1585 of Dr. Phillip Berman. The experiments disclosed here focus on, among other things, the further development of the ELISA developed previously for identifying human herpes antibodies using the gD12 cell line. *See supra*

at 16. Noteworthy, was the success of the ELISA using human sera obtained from the Center for Disease Control (CDC) disclosed at pages 34-45 and 49-53. Dr. Berman also obtained successful immunoprecipitations of culture supernatants from the 10.2 CHO cell line. Immunoprecipitations against the truncated version of gD were performed throughout these pages using both polyclonal and monoclonal antibodies. As indicated on page 13, Dr. Berman concluded that the secreted truncated gD protein possessed at least one neutralizing antigenic determinant. Pages 55-56, 60-61, and 83-85 disclose Dr. Berman's experiments regarding the post translational modification of the membrane bound gD protein. The experiments included pulse-chase labeling of gD12 CHO cells with ^{35}S-methionine over time.

18. Appended DOCUMENT 10 includes pages 1-3, 9, 18-28, 32-39, 41-48, 62-63, 67-68 and 72-75 of notebook 1705 of Dr. Phillip Berman. These pages disclose Dr. Berman's continued refinement of the gD12 cell based ELISA. In addition notebook pages 32-33, 38, 43-48, 54 and 62-63 disclose further experiments wherein Dr. Berman was immunoprecipitating ^{35}S-methionine or ^{35}S-glucosamine labeled gD12 or 10.2 CHO cells. Dr. Berman, at pages 1-3, 9 and 41 continued examining fluorescent labeling of these cell lines as well as investigating immunoprecipitations of gD products from various HSV-1

infected cell lines compared with that of the gD12 cell line.. As shown at pages 67-68 and 72-75 these cell lines included A549, Vero and HEL.

19. Appended DOCUMENT 11 includes pages 6, 21-22 and 28-30 of notebook 1884 of Dr. Phillip Berman. These pages disclose further experiments investigating indirect immunofluorescence studies of gD producing CHO cell lines as well as Dr. Berman's development of a gD12 based competitive binding assay for quantifying the relative amounts of truncated gD secreted into culture medium by CHO cells before and after selection in methotrexate.

20. Appended DOCUMENT 12 includes pages 7-9, 15, 17-18, 23-25, 27-35, 37, 43-48, and 50-54 of notebook 1501 of Dr. Phillip Berman. These pages disclose immunization and HSV challenge studies of rabbits and mice injected with either the full-length membrane-bound form of gD as found on the surface of gD12 CHO cells or the secreted, truncated form of gD as produced by 10.2 CHO cells. For example pages 8-9 disclose immunizing rabbits with gD12. As disclosed, this work was begun in January, 1983.

21. Pages 7, 15, 17-18 and 23-24 disclose mouse immunization protocols 454 and 434. Under protocol 454, disclosed at page 15, it was recorded that one group of animals, female Balb/C mice, were immunized with gD cells producing membrane bound gD. Likewise,

protocol 434, found on page 17, discloses that one group of female Balb/C mice, group G, was again injected with gD12 cells. Both of these experiments were performed to see whether mice immunized in this fashion developed antibodies to the membrane bound gD.

22. Noteworthy are the experiments beginning at page 30 disclosing immunizing mice with the secreted, truncated form of the gD protein from 10.2 CHO cells. This experiment was part of mouse protocol 509. *See infra at* 25. As disclosed at page 35, this experiment began in May, 1983. 8-week old Balb/C mice were immunized with 10.2 CHO cell produced gD protein (Group C) while control mice were injected with human serum albumin (HSA) (Group D). These animals were boosted subsequently as indicated. As disclosed in Lasky notebook 1915, after a subsequent boost, sera was collected from these animals and tested for in vitro neutralizing capability. *See supra at* 11.

23. More importantly, as indicted on page 35, Group C and D mice were challenged, in vivo, with HSV-1 beginning July, 1983. As the data indicates, by 21 July 1983 all Group C immunized mice remained healthy while all but 3 of the control Group D mice had died or were dying. Dr. Berman concluded that "These results show unequivocally that the secreted truncated gD can provide protection from HSV-1 infection".

24. Equally important is the experiment, beginning on page 37 and continuing at pages 43, 47, and page 50 regarding mouse protocol 527. *See infra at* 26. Here, 5-week old Balb/C mice were either immunized with gD12 CHO cells or simply CHO cells alone in June, 1983. These same mice were boosted in July and August, 1983. Page 51 indicates that 10 mice from the immunized group (Group A) and 10 mice from the control group (Group B) were challenged, in vivo, with HSV-1 virus beginning 16 August 1983. Prior to this, sera obtained from these mice were submitted to Dr. Lasky for in vitro neutralization assays. As discussed previously, these assays confirmed that neutralizing antibodies were being produced by the immunized mice. *See supra at* 12. As indicated below, the in vivo challenge showed that all mice in the control group died within 7 days of infection while those in the immunized group showed protection and no evidence of infection. *See infra at* 26. Thus, as with the truncated protein, immunization with the gD12 cells protected mice, in vivo, from a lethal HSV-1 challenge.

25. Appended DOCUMENT 13 includes notebook pages from Berman notebook 1501 and an animal protocol record sheet from protocol number 509. As discussed previously, this protocol concerned immunizing mice with secreted gD protein from 10.2 CHO cells and eventually challenging them, in vivo, with HSV-1 virus. Challenge

results revealed that immunized mice (10) were fully protected against the lethal HSV-1 infection whereas by 21 July 1983, 7 mice from the control group were dead while 3 were dying as indicated in Dr. Berman's notebook 1501 at page 35 *See supra at* 22,23.

26. APPENDED DOCUMENT 14 represents animal protocol 527. As discussed previously, this protocol provided that mice be either immunized and boosted with gD12 cells producing membrane bound gD or injected and boosted with just CHO cells. *See supra at* 24. As disclosed on the second and third pages of the document, the mice, 10 in each group were challenged, in vivo, with HSV-1 virus on 16 August 1982. As disclosed on the fourth and fifth pages of the document, the animal protocol record sheet, by 25 August 1983 animals 1-10 (immunized with gD12 cells and challenged with HSV-1) had survived the challenge whereas control animals 21-30 (injected with CHO cells and challenged with HSV-1) were dead. Drs. Lasky and Berman concluded that mice injected with cells producing a membrane bound gD would elicit antibodies capable of fully protecting the mouse from a lethal challenge.

27. We hereby declare that all statements made herein of our knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are

punishable by and imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issue thereon.

Dated: 10/18/95

Laurence A. Lasky

Dated: 10/18/95

Phillip W. Berman